

Courtship Behavior of *Bemisia argentifolii* (Hemiptera: Aleyrodidae) and Whitefly Mate Recognition

THOMAS M. PERRING AND EMILY J. SYMMES

Department of Entomology, University of California, Riverside, Riverside, CA 92521

Ann. Entomol. Soc. Am. 99(3): 598–606 (2006)

ABSTRACT *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) consists of a species complex with various degrees of reproductive compatibility between biotypes. One mechanism known to result in reproductive isolation among sexually reproducing animals is mate recognition. Whiteflies have an elaborate courtship and mating behavior, and it is well known that individuals of some biotypes in the *B. tabaci* species complex will court individuals of other biotypes, but they will not mate. In this study, we determined specific courtship and mating behaviors of *B. tabaci* biotype B (*Bemisia argentifolii* Bellows & Perring). Four distinct phases in the courtship and mating cascade were identified, and we describe these phases and report their durations. We compared our findings with previously reported mating behaviors of two other whiteflies, *B. tabaci* biotype A, and *Trialeurodes vaporariorum* (Westwood). These comparisons identified both similarities and differences in the behaviors of the three whiteflies, particularly in the extent and position of antennal drumming, male abdominal undulation, and wing and body position during copulation. Body pushing behavior, characterized for *B. tabaci* biotype A and *T. vaporariorum*, was not present for *B. argentifolii*. The similarities between whiteflies may represent evolutionarily conserved behaviors, resulting in courtships between reproductively incompatible whiteflies. Conversely, differences in behaviors may contribute to prezygotic reproductive isolation among biotypes. From our studies, we propose that the discrimination of signals sent and received from courting whiteflies becomes more intense with each successive phase in the courtship cascade.

KEY WORDS whitefly, *Bemisia tabaci* biotype A, *Trialeurodes vaporariorum*, behavior

The sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), has emerged in recent years as one of the most formidable agricultural pests in the world. It is known to exist in all major agricultural regions (Cock 1986, 1993), and its distribution has been advanced by human trade activities (Brown et al. 1995b, DeBarro 1995). Originally described as *Aleyrodes tabaci* Gennadius (Gennadius 1889), *B. tabaci* currently exists in synonymy with 22 other species (Mound and Halsey 1978), based largely on the morphology of the fourth instar. A result of these synonymizations is that *B. tabaci* populations from distinct geographic locations have tremendous variation in their biological characteristics (e.g., host range, geographic distribution, life stage information, behavior, virus-vectoring capacity, interactions with natural enemies, response to chemicals; Oliveira et al. 2001).

In an effort to provide some understanding of the variation among populations, Brown et al. (1995b) and Frohlich et al. (1999) proposed *B. tabaci* as a species complex, with distinct populations referred to as biotypes. Currently, there are 19 biotypes that are named, based on nonspecific esterase banding patterns (Bedford et al. 1992; Brown et al. 1995a, 2000; DeBarro et

al. 2000; Simon et al. 2003), and other biotypes associated with various hosts or various geographic locations have been reviewed by Perring (2001). Particularly interesting is the degree of reproductive compatibility between various biotypes. For example, Costa et al. (1993) and Perring et al. (1993) crossed biotypes A and B from the United States and reported a lack of F1 females (suggesting a lack of gametic transfer in the haplodiploid *B. tabaci*). Based on these results and further distinctions at the genetic level, Perring et al. (1993) proposed biotypes A and B represent two distinct species, and Bellows et al. (1994) provided a species description for biotype B, designating it *Bemisia argentifolii* Bellows & Perring. Depending on individual's definition of "species," some researchers accept this new species, others use it within a cryptic species concept, and others reject it, preferring to use biotype B nomenclature. In the present discussion, it is not important to which taxonomic level the B biotype is elevated, only that it is reproductively incompatible with other biotypes. Studies with this and other *B. tabaci* biotypes provide further evidence of reproductive incompatibility among members of the species complex. Bedford et al. (1994) performed reciprocal crosses between biotype

B from the United States, biotype K from Pakistan, biotype M from Turkey, and biotype D from Nicaragua and found that none of the biotypes interbred with other biotypes. Recently, mating incompatibility was documented among three allopatric *B. tabaci* populations on cassava *Manihot esculenta* Crantz (from India, Tanzania, and Uganda), between two sympatric populations from Uganda on different host plants (cassava and sweet potato *Ipomoea batatas* (L.) Lam), and between two sympatric populations from India on different host plants (cassava and *Euphorbia geniculata* Ortega) (Maruthi et al. 2004).

Compatible crosses among *B. tabaci* biotypes have been documented as well. Hybrids have been identified in crosses between biotype B (United States) and biotype L (Sudan) (Byrne et al. 1995). Similar results were recorded by Gunning et al. (1997) between a native Australian population and biotype B and between biotypes B and Q in Spain (Ronda et al. 2000). DeBarro and Hart (2000) found F1 hybrids were produced among two Australian populations and the B biotype. Although hybrids were produced in all these studies, the authors did not indicate whether the F1 hybrids were fertile. DeBarro and Hart (2000) reported that the hybrids produced in their studies were sterile. Contrary to these findings, Brown et al. (2001) reported fertile F1 hybrids between biotype A, biotype B, and the *Jatropha* biotype, and fertile hybrids between biotypes B and Q have been reported from Spain (Francisco J. Beitia, personal communication).

Summarizing these studies, it is clear that various levels of reproductive compatibility are operating in the *B. tabaci* species complex. Several mechanisms could result in this isolation. Nirgianaki et al. (2003) found variation in the presence of *Wolbachia* between whitefly biotypes, suggesting that the presence of *Wolbachia* may play a "role in the evolution of some of these biologically and genetically distinguishable populations and biotypes." Prezygotic mechanisms also can drive reproductive incompatibility, and one such mechanism that may be operational in the *B. tabaci* species complex is the mate recognition system. Butlin (1995) noted that divergence in mating signals and responses can be an important step in the speciation of sexually reproducing animals and may precede the evolution of complete postmating isolation. This "sexual or psychological isolation" was discussed by Dobzhansky (1937) as a mechanism preventing hybridization between two sympatric parental types. Petit-Marty et al. (2004) further wrote that sexual behavior is "the key to define a biological species."

Interestingly, several researchers observed that although males and females of distinct *B. tabaci* populations did not mate, they engaged in extensive courtship behavior (DeBarro and Hart 2000; Maruthi et al. 2004; T.M.P., unpublished data). These whiteflies may share some behaviors, yet lack the necessary signals that result in mating between the variants. Were this true, it would support the statement of Butlin (1995) that "while closely related species tend to use similar mating signals, they almost always have distinct signals." The courtship and mating behavior of *B. tabaci*

biotype A has been described in detail (Li et al. 1989) as have the behaviors of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Las 1979, Ahman and Ekblom 1981, Li and Maschwitz 1985). In the current article, we describe the courtship behavior of *B. argentifolii*. The behaviors of these three whiteflies then were compared in an effort to deepen our understanding of whitefly mating behavior.

Materials and Methods

Our *B. argentifolii* colony was established with several thousand individuals collected from broccoli *Brassica oleracea* L. fields in California's Imperial Valley (Riverside County) in 1991 (Perring et al. 1992). This colony was maintained at high densities on lima bean, *Phaseolus lunatus* L. 'Fordhook', and mustard, *Brassica juncea* (L.), in a greenhouse under natural daylength at $27 \pm 4^\circ\text{C}$ from 1991 to 2002. In 2002, the colony was moved to an environmentally controlled insect rearing room with four 32-Watt fluorescent bulbs set at a photoperiod of 14:10 (L:D) h. Over the course of the current experiments, the average temperature in the rearing cage was 26.6°C (range $25.95\text{--}27.91^\circ\text{C}$) with a relative humidity of 26.6% (range 17.4–37.3%). Temperature and humidity data were collected using a Hobo data logger (model H8 Pro Series, Onset Computer Corporation, Bourne, MA).

To obtain whiteflies for mating experiments, lima bean leaves containing numerous fourth instars were excised from the colony plants. The cut ends of the leaf petioles were wrapped in wet cotton to maintain leaf moisture until whitefly eclosion. After all adult whiteflies were removed from the leaves, each leaf was placed individually in a plastic petri dish (14 cm in diameter, 2 cm in depth) until adult emergence. Adult whitefly transfers were accomplished by placing the petri dishes containing leaves and whiteflies in a freezer until they were immobilized, at which point they were removed from the freezer and transferred using a camel's-hair brush. Adults were removed from the petri dish within 12 h of eclosion, ensuring virginity of the whiteflies. Perring et al. (1993) reported that *B. tabaci* biotype B females isolated from males within 12 h after eclosion produced only F1 males, indicating that they had not mated. Whiteflies were separated by gender and placed in holding arenas that were created by pressing a piece of 0.9-cm-thick acrylic plastic with a 3-cm-diameter hole bored in the middle against the abaxial surface of a clean excised lima bean leaf. These excised leaves also were kept moist by placing wet cotton around the cut end of the petiole. The arena was covered with a glass coverslip. When sufficient numbers were collected to conduct a trial (≈ 24 h after setting up the emergence petri dish) two or three pairs of males and females were placed together in an observational arena. These arenas were made from 0.6-cm-thick acrylic with a 0.8-cm-diameter hole (for three pairs) or a 0.5-cm-diameter hole (for two pairs) pressed to the abaxial surface of a clean

bean leaf. Again, these excised leaves were kept moist by placing wet cotton around the cut end of the petiole. A glass coverslip was used to cover each arena and facilitate videorecording.

Whitefly behaviors were recorded at room temperature using a Leica microscope (model Wild MZ28, Meyer Instruments, Houston, TX), equipped with a Panasonic digital camera (model WV-CP410, Matsushita Communication, Osaka, Japan) connected to a Sony video monitor (model PVM-14N1U, Sony Corporation, Surrey, United Kingdom) and a Lorex time-lapse videocassette recorder (model SG7924R, Strategic Vista Corporation, Ontario, Canada). A 115-volt Nikon fiber optic light (model MK, Nikon, Inc., Garden City, NY) was used to illuminate the mating arena for the recordings. The lights were maintained at a constant setting throughout all video tapings. Mating behavior was recorded at normal speed onto standard 6-h videotapes, and each group of whiteflies placed into the mating arenas was recorded for a single 6-h period.

B. argentifolii males and females may mate multiple times in their lifetime (T.M.P., unpublished data), and this was observed periodically in our study. Therefore, to eliminate variation resulting from differences in sexual behavior of mated versus nonmated whiteflies, data were collected only on courtship sequences between virgin males and virgin females. In total, 144 h of behavior from 132 courtships was recorded, the videotapes were replayed and observed, and mating behaviors and durations of courtship sequences were documented.

After analyzing data from our experiments, the courtship behaviors were compared with the results on *B. tabaci* (Li et al. 1989) and *T. vaporariorum* (Las 1979, Ahman and Ekblom 1981). The concept of biotypes had not been applied to *B. tabaci* when Li et al. (1989) conducted their studies; therefore, they did not define the biotype on which they worked. However, they completed their work in 1982 (D. Gerling, personal communication), before the arrival of biotype B in the United States (Price et al. 1987), and aside from biotype B, biotype A is the only other whitefly variant that has been identified from the United States (Brown et al. 1995a, Perring 2001). Therefore, we conclude that Li et al. (1989) was working on *B. tabaci* biotype A.

Results

Before courtship begins, the males and females must come into contact with each other. For *B. argentifolii*, the males made initial contact with the females; we did not observe females initiating contact with males. Males used their antennae (68% of the encounters) or their tarsi (19% of the encounters) to touch the females. In the remaining 13% of the encounters, it was unclear what body part the male used to initiate contact with the female. Primary contact was made to the female wing (62% of encounters), thorax (13%), antenna (10%), tarsus (1%), or head (1%), and the fe-

male body part initially contacted was unclear in the remaining 13% of the encounters.

After initial contact, 91% of the males maintained constant contact with the females through the orientation process by using their antennae, tarsi, or both, and they quickly moved into parallel orientation with females. In 72% of the encounters, they oriented on the same side of the female as the initial contact; however, there was some variation in their movements before becoming parallel. When initial contact was to the posterior end of the female, 37% of the males moved directly up the side of the female until they reached the parallel position. When initial contact was to the anterior region of the female, the male moved down and back up the side of initial contact and this occurred in 28% of the encounters. In 7% of the encounters, males traveled the length of the female two times before settling in parallel orientation, again on the side of the initial contact. Sometimes (19% of the encounters) males circled the females, taking up parallel orientation on the side opposite the initial contact. These males made half-circles on the anterior end (7%), half circles on the posterior end (5%), or complete circles of the females (7%). Male positioning in the remaining 9% of the encounters was highly variable and we were unable to categorize them by any single behavior or behavioral sequence.

Phase 1: Antennal Drumming. Immediately after parallel orientation, males began antennal drumming. The middle part of the male antenna closest to the female was used to drum the middle part of the nearest female antenna. Occasionally, males also drummed the head, thorax, or nearest foreleg of the female. Phase 1 is defined as the period after parallel orientation, from the time males initiated antennal contact with females until antennal drumming ceased. In *B. argentifolii*, this initial orientation stage of courtship lasted an average of 27 s with a duration range of 5–90 s (Table 1). Of the pairs entering phase 1 ($n = 132$), 91.7% advanced to phase 2, 2.3% advanced to phase 4, and 6% ended courtship behavior (Fig. 1). This termination of the courtship was determined by pairs ending parallel orientation behavior.

Phase 2: Male Abdominal Undulation. Once antennal drumming ended, males eventually began abdominal undulation, in which the male abdomen moved up and down in a vertical plane, apparently contacting the leaf surface with each undulation. Occasionally, female abdominal undulation behavior was observed as well. Infrequently, males were observed “flicking” their wings during this phase. These wing flicks involved short intermittent periods of wing movement in which the wings were quickly raised and lowered vertically above the male’s abdomen or at a slight angle above the abdomen of the female. Wing flicks at this point did not coincide with copulation or copulation attempts, and they generally were responses by the male to interference by other whiteflies in the arena or to “refusal behavior” by the female. Movements identified as refusal behaviors by females included grooming, wing flicking, and attempting to leave the parallel courtship orientation. Often, male abdominal

Table 1. Duration of courtship phases of *B. argentifolii*

Phase ^a	No. entering phase ^b	No. completing phase ^c	Duration range (s)	Avg duration (s) ± 95% CI
1	132	124	5–90	27 ± 3
2	190	88	8–1559	370 ± 61
3	0	0		
4	90	85	1–77	3 ± 2
C	16	16	131–370	181 ± 27

^a Refer to text for phase descriptions.
^b Number of courting pairs that entered the phase. Some pairs entered phases multiple times; therefore, the number of pairs in phase 2 exceeds the number of pairs that originally entered phase 1.
^c Number of courting pairs that completed the phase.

undulations were not continuous throughout the entire length of phase 2 and became intermittent or stopped completely during this phase. The duration of phase 2 was recorded from the cessation of male antennal drumming until the male raised all four wings, partially covering the wings of the female and positioning his abdomen beneath hers in preparation for copulation. Phase 2 in *B. argentifolii* ranged in duration from 8 to 1559 s with an average of 370 s (Table 1). Phase 2 was entered by 190 mating pairs with some pairs entering this phase multiple times. Of these 190 pairs, 53.7% terminated courtship during phase 2, 0.5% reverted to phase 1, and the remaining 45.8% proceeded to phase 4 (Fig. 1).

Phase 3: Body Pushing. This phase was included in the discussion here because it was observed in previous studies of the courtship behaviors of *B. tabaci* biotype A (Li et al. 1989) and *T. vaporariorum* (Las 1979, Ahman and Ekblom 1981, Li and Maschwitz 1985). Body pushing behavior was not observed in the courtship sequence of *B. argentifolii* in the current study.

Phase 4: Male Positioning. Phase 4 in *B. argentifolii* began when the male raised all four wings, partially covering the wings of the female. During this phase, the male oriented his abdomen below the female's

abdomen, at a 35–45° horizontal angle to her abdomen. This phase lasted until the pair initiated copulation, reverted to courtship with male abdominal undulations (phase 2), or terminated courtship. The duration of phase 4 averaged 3 s (range 1–77 s; Table 1). Of the *B. argentifolii* pairs entering phase 4 (*n* = 90), 17.8% achieved complete and prolonged organ contact and were documented as reaching the copulation phase (phase C), 76.7% returned to phase 2, and the remaining 5.5% terminated courtship (Fig. 1).

Phase C: Copulation. Phase C represents successful copulation. This phase began when the tip of the male abdomen contacted the tip of the female abdomen and ended after copulation when the abdomens no longer were in contact with each other. Phase C in *B. argentifolii* lasted an average of 181 s, with a range of 131–370 s (Table 1). The proportion of successful copulations resulting from initial courtship encounters in *B. argentifolii* was 12% (16/132), which compares with 7.7% reported for *B. tabaci* biotype A (Li et al. 1989). These low success rates may be the result of a high degree of signal selection between *Bemisia* sp. males and females that must be satisfied through a complex courtship mechanism. The majority (81.3%) of pairs that copulated went directly from phase 1 to phase 2 to phase 4 a single time, whereas pairs that reverted to

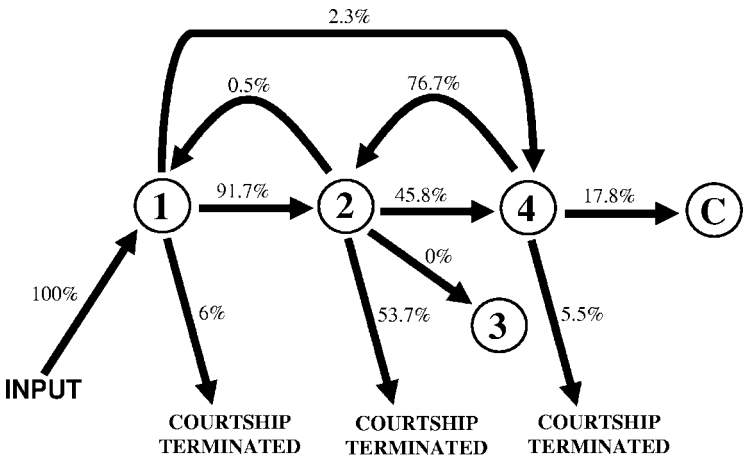


Fig. 1. Sequence of observed courtship behaviors of *B. argentifolii*. Numbers 1, 2, 3, 4, and letter C refer to phases in the courtship as defined in the text. Proportions shown were calculated from the number of mating pairs that entered the phase (Table 1) and the number of pairs that continued to a subsequent phase, returned to a previous phase, or terminated courtship behavior.

phase 2 multiple times accounted for only 18.7% of the successful copulating pairs.

After copulation, the pair remained in parallel orientation for variable lengths of time, between 5 s and >18,030 s (when the 6-h tape was completed). There was no apparent courtship interaction between the male and female during this period, and they were observed feeding and grooming themselves. In one case, a pair copulated a second time, 5,885 s after the first copulation ended. The behaviors leading to this second copulation, and the times of these behaviors were not included in previous analyses.

Unsuccessful Courtship Sequences. Terminated courtship sequences in *B. argentifolii* were identified as the complete departure from parallel orientation by either the male or the female of the courting pair. Of the total aborted courtship sequences ($n = 116$), 66.4% were terminated due to females moving away from males, whereas 32.7% ended when males left the mating orientation. Simultaneous rejection by both sexes occurred in one of 116 unsuccessful courtships (0.9%). These frequencies suggest that females are more discriminating when selecting a mating partner than are males. During phase 1, 75% of terminated courtship sequences were the result of female departure and in phase 2 female termination accounted for 72% of discontinued mating behavior. During phase 4 and into copulation attempts, males and females were equally responsible for ending the mating sequence. In pairs that had reverted back to phase 2 after reaching phase 4, females ended the courtship 42% of the time, and males were responsible for 58% of the aborted courtship sequences at this point in the mating sequence.

Male Interference of Courtship. Las (1979), Ahman and Ekblom (1981), and Li et al. (1989) reported that courting pairs in their studies sometimes were interrupted by single males. We have observed this behavior for *B. argentifolii*, thus we analyzed the frequency and impact of this behavior in our studies. Male interference occurred 17 times out of the 132 courtships that we recorded. Usually, the first contact from the intruding male was with the courting female; however, occasionally the intruding male made initial contact with the primary courting male. In all cases, the intruding male spent little time touching the courting whiteflies, and he quickly moved toward parallel orientation on the "free" side of the courting female. During this orientation attempt, the primary courting male flicked all four wings vertically or flicked the two wings nearest the female, covering the female with his wings, and touching the intruding male with his extended wings. Once the intruding male was successfully in parallel orientation, the three whiteflies seemed to be still, with intermittent "bursts" of wing flicking by one or both of the males.

The consequence of male interference was a complete lack of copulation between the female and either of the competing males. In eight of the 17 cases, the female left the courtship, leaving both males in parallel orientation; in once case, the intruding male antennated the primary courting male. In this case, the males remained in parallel orientation for a short time

before the primary male departed; there were no abdominal undulations when the two males were in parallel orientation. In six of the 17 cases, the intruding male was "successful" in maintaining the courtship as the primary courting male left the female; this never resulted in copulation. In the remaining three of the 17 cases, the primary courting male was successful in maintaining the courtship when the intruding male left; again, this courtship never resulted in copulation. Our studies indicate that it is counterproductive for multiple males to court the same female, because these courtships result in the absence of mating, and they unproductively use courtship time of both males and females.

Comparing Behaviors among Whiteflies. Analyzing the mating behaviors of *B. argentifolii*, *B. tabaci* biotype A (Li et al. 1989), and *T. vaporariorum* (Las 1979, Ahman and Ekblom 1981, Li and Maschwitz 1985) revealed similarities and differences. Although direct comparisons of all phases and phase durations for all three whiteflies are not possible due to differences in definitions of the courtship phases for each, certain behaviors can be compared (Table 2). For example, male antennal drumming of female antennae in *B. argentifolii* did not continue after male abdominal undulation began. In the other two whiteflies, male antennal drumming lasted through the abdominal undulation phase until males raised their wings for copulation (*B. tabaci* biotype A) or until the males "bumped" the females just before copulation (*T. vaporariorum*). Antennal drumming position of *B. argentifolii* was found to be similar to that of *B. tabaci* biotype A, in which males used the middle portion of the antenna nearest the female to drum the middle portion of the nearest female antenna. This position was very different from the antennal drumming position described for *T. vaporariorum*. Male *T. vaporariorum*, once in parallel orientation with females, used the antenna nearest the female to drum her thorax while using the antenna farthest from the female to drum the underside of the distal end of the closest female antenna (Las 1979). Abdominal undulation behavior by males was found to occur in all three species, but to varying extents. Males of *B. argentifolii* began abdominal undulations after antennal drumming behavior had stopped completely. Abdominal undulation behavior by males of *B. tabaci* biotype A and *T. vaporariorum* occurred in synchrony with antennal drumming (Ahman and Ekblom 1981, Li et al. 1989).

Perhaps the most variable behaviors exhibited by the three whiteflies were wing "blocking" and "bumping" or body pushing (Table 2). Wing blocking, described by Ahman and Ekblom (1981) as a period of high wing flick frequency, followed by inactive periods for *T. vaporariorum*, was absent in the two *Bemisia* whiteflies. Body pushing, where males pushed the sides of female causing the pair to move in a semicircular pattern with their heads toward the center, was found in <15% of the *B. tabaci* biotype A courtships (Li et al. 1989). A more vigorous type of pushing, referred to as "bumping" was found in *T. vaporariorum*

Table 2. Comparisons of courtship and mating behavior of *Bemisia argentifolii*, *Bemisia tabaci* biotype A, and *Trialeurodes vaporariorum*

Behavior	<i>B. argentifolii</i>	<i>B. tabaci</i> ^a	<i>T. vaporariorum</i> ^b
Male antennal drumming	Antennal drumming ceased before male abdominal undulation began.	Antennal drumming lasted until male raised wings for copulation attempt.	Antennal drumming lasted until "bumping" of female by male. This was noted as a precopulation stage.
Antennal drumming position	Male used middle portion of antenna nearest female to drum middle portion of nearest female antenna. Occasional drumming of female head, thorax, or nearest leg.	Male used antenna nearest female to drum middle portion of nearest female antenna.	Male used antenna nearest female to drum her thorax. Distal male antenna was used to drum the underside of the nearest female antenna. His antennae "scissor" where male appeared to be rubbing female antennae.
Abdominal undulation	Male abdominal undulation and antennal drumming occurred at distinct times during separate courtship phases.	Male moved abdomen up and down in synchrony with antennal drumming, gradually increasing speed.	Males moved abdomen up and down, apparently in time with the antennal strokes.
Wing "blocking"	Not observed.	Not observed.	Blocks, which are periods of high wing flick frequency followed by periods of inactivity, were used to distinguish stage 3 (Ahman and Ekblom 1981).
"Bumping" or body pushing	Not observed.	While males hasten the tempo of antennal drumming, they push female with the side of their body, causing the pair to move in a semicircular pattern with their heads toward center. Observed in <15% of courtship sequences.	After antennal drumming ceased, males vigorously bumped female thorax with forelegs and head, resulting in lateral repositioning of the pair. Observed in all courtship sequences.
Wing position during copulation	Male raised all four wings to cover female's wings.	Male raised only the pair of wings nearest the female to cover the female's wings.	Males raised all four wings to cover female's wings.
Body angle between male and female during copulation	35–45°	24°	35°

^a From Li et al. (1989).
^b From Las (1979), Ahman and Ekblom (1981), and Li and Maschwitz (1985).

courtships (Las 1979). Li et al. (1989) referred to these behaviors as "essential" for *T. vaporariorum* and "non-essential" for *B. tabaci* biotype A. However, neither of these behaviors was observed in our studies of *B. argentifolii*.

Wing position during copulation and copulation attempts also varied between the three whiteflies. In the copulation phase of *B. argentifolii*, the male raised all four wings, partially covering the female's wings, and oriented himself at an angle to the female. This wing and body position of male *B. argentifolii* during copulation was nearly identical to the position described for *T. vaporariorum* during this stage of the mating sequence (Li and Maschwitz 1985). In contrast, Li et al. (1989) found that male *B. tabaci* biotype A raised only the pair of wings nearest the female to cover the female's wings during copulation. In addition, male *B. tabaci* biotype A oriented themselves at an $\approx 25^\circ$ angle to the females during copulation (Li et al. 1989), as opposed to the $\approx 35^\circ$ angle that was noted for *T. vaporariorum* (Li and Maschwitz 1985) and the $35\text{--}45^\circ$ angle recorded for *B. argentifolii* in the current study.

Discussion

Courtship behavior serves a number of functions, one of which is to assist in the recognition of mates as belonging to the appropriate gender and species. One theory likens insect courtship to a ratchet mechanism, in which the courtship sequence progresses in a sequential manner where each step leads to the next and courtship must progress in a precise order to achieve successful copulation (Matthews and Matthews 1978). This theory proposes that if certain courtship behaviors are not exhibited, or if the sequence is not followed, this chain reaction mechanism serves to prevent mating which Butlin (1995) notes can lead to reproductive isolation. Our study of *B. argentifolii* supports this ratcheting-type courtship cascade, and further suggests an intensification of discrimination in signals between the sexes with each successive step in the cascade.

The most successful courtship behavior, defined as the highest proportion of pairs completing the behavior, was phase 1. This phase was completed 124 times by the 132 courtship pairs entering this phase ($94\% = 91.7\%$ proceeding to phase 2 and 2.3% proceeding to phase 4). This phase averaged 27 s in duration, and had low variability relative to the other phases, suggesting that signal discrimination during this phase may have been weak relative to the other phases.

In phase 2 (abdominal undulation), 45.8% of the whitefly pairs that entered this phase completed it, 53.7% terminated courtship, and 0.5% returned to phase 1. The high proportion of courtships that ended during this phase indicates a more selective signal discrimination between courting pairs than occurred during phase 1 (6% termination). Supporting this, there was substantial variation in the duration of this phase (ranging from 8 to 1,559 s), and we discovered that when two males were in parallel orientation after

the female over which they competed left the courtship, they did not engage in abdominal undulation behavior.

The next phase in the *B. argentifolii* courtship cascade was body positioning (phase 4). This phase lasted an average of only 3 s, and a small proportion (5.5%) of pairs that reached phase 4 terminated courtship. This low proportion may result from pairs having already completed the majority of the courtship cascade during which they have continued sending and receiving appropriate signals to and from their prospective mate. However, mating was not eminent at this phase; in fact only 17.8% of the courting pairs that entered phase 4 moved into copulation. This compares to 23% reported for *B. tabaci* biotype A (Li et al. 1989). According to Li et al. (1989), during this phase the male claspers open, allowing the aedeagus to protrude while the male attempts to clasp the female terminalia (Las 1979, Li and Maschwitz 1985, Li et al. 1989). Copulation ensues after the female allows the male to pull down the terminal flap that covers her gonopore and insertion of the aedeagus (Las 1979, Li and Maschwitz 1985). In the current study, the largest proportion (76.7%) of whiteflies that reached this phase reverted back to abdominal drumming (phase 2) and additional courtship discrimination. This reversion was repeated numerous times and could be responsible for the large variation that is present in the overall courtship times of mating *B. argentifolii* (T.M.P., unpublished data). In the same way that abdominal drumming (phase 2) represents an early signal discrimination phase, we propose that body positioning (phase 4) is the final discriminating point before copulation.

Considering the information on the three whiteflies discussed in this article, some of the behaviors had only slight variations between the whitefly types (duration of antennal drumming, synchronous abdominal undulations, body angle between male and females during copulation), whereas other behaviors were unique (i.e., antennal drumming position in *T. vaporariorum*, absence of body pushing behavior in *B. argentifolii*, only two wings cover the female abdomen in *B. tabaci* biotype A). The fact that whiteflies, even in distinct genera, have similar behaviors helps explain the observation that males and females of reproductively incompatible whiteflies in the *B. tabaci* species complex will invest significant time courting each other (DeBarro and Hart 2000; Maruthi et al. 2004; T.M.P., unpublished data). Behavioral differences between biotypes, however, represent critical junctions in the sending and receiving of signals between sexes. Our data support the hypothesis that signal discrimination becomes more intense as the mating cascade progresses. This discrimination may serve to prohibit successful mating, essentially providing a prezygotic mechanism leading to reproductive incompatibility among whitefly types. It would be interesting to study the mating behaviors of other *B. tabaci* biotypes to see whether this mechanism is operational throughout the species complex.

Acknowledgments

We thank Rosalyn Goh for many hours in the laboratory taping and recording whiteflies. We also thank Chuck Farrar, Justin Nay, Yong-Lak Park, and Rayda Krell for critical comments on an earlier draft of the manuscript. This research was supported, in part, by the Ventura County Whitefly Action Committee and the California Strawberry Commission.

References Cited

- Ahman, I., and B. S. Ekblom. 1981. Sexual behavior of the greenhouse whitefly (*Trialeurodes vaporariorum*): orientation and courtship. *Entomol. Exp. Appl.* 29: 330–338.
- Bedford, I. D., R. W. Briddon, P. G. Markham, J. K. Brown, and R. C. Rosell. 1992. *Bemisia tabaci* - biotype characterization and the threat of this whitefly species to agriculture. *Proc. 1992 Br. Crop. Prot. Conf. Pests Dis.* 3: 1235–1240.
- Bedford, I. D., P. G. Markham, J. K. Brown, and R. C. Rosell. 1994. Geminivirus transmission and biological characterization of whitefly (*Bemisia tabaci*) biotypes from different world regions. *Ann. Appl. Biol.* 125: 311–325.
- Bellows, T. S., Jr., T. M. Perring, R. J. Gill, and D. H. Headrick. 1994. Description of a species of *Bemisia* (Homoptera: Aleyrodidae). *Ann. Entomol. Soc. Am.* 87: 195–206.
- Brown, J. K., R. Caballero, D. Rogan, and J. Bird. 2001. Evidence for *Bemisia tabaci* species complex: mitochondria cytochrome oxidase I gene complex sequence analysis confirms one group comprising all *B. tabaci*, and mating between AZA, AZB and Jatropa biotypes corroborate a single biological species, pp. 23. *In* Proceedings, European Whitefly Symposium, 27 February–3 March 2001, Ragusa, Sicily, Italy. European Whitefly Studies Network, Norwich, United Kingdom.
- Brown, J. K., S. A. Coats, I. D. Bedford, P. G. Markham, J. Bird, and D. R. Frohlich. 1995a. Characterization and distribution of esterase electromorphs in the whitefly, *Bemisia tabaci* (Genn.) (Homoptera, Aleyrodidae). *Biochem. Gen.* 33: 205–214.
- Brown, J. K., D. Frohlich, and R. Rosell. 1995b. The sweet-potato/silverleaf whiteflies: biotypes of *Bemisia tabaci* (Genn.), or a species complex? *Annu. Rev. Entomol.* 40: 511–534.
- Brown, J. K., T. M. Perring, A. D. Cooper, I. D. Bedford, and P. G. Markham. 2000. Genetic analysis of *Bemisia* (Homoptera: Aleyrodidae) populations by isoelectric focusing electrophoresis. *Biochem. Genet.* 38: 13–25.
- Butlin, R. 1995. Genetic variation in mating signals and responses, pp. 327–366. *In* D. M. Lambert and H. G. Spencer [eds.], *Speciation and the recognition concept, theory and application*. The Johns Hopkins University Press, Baltimore, MD.
- Byrne, F. J., M. Cahill, I. Denholm, and A. L. Devonshire. 1995. Biochemical identification of interbreeding between B-type and non B-type strains of the tobacco whitefly *Bemisia tabaci*. *Biochem. Genet.* 33: 13–23.
- Cock, M. J. W. 1986. *Bemisia tabaci*, a literature survey on the cotton whitefly with an annotated bibliography. CAB International Institute of Biological Control, Caneelton Press, London, United Kingdom.
- Cock, M. J. W. 1993. *Bemisia tabaci*, an update 1986–1992 on the cotton whitefly with an annotated bibliography. CAB International Institute of Biological Control, Ascot, United Kingdom.
- Costa, H. S., J. K. Brown, S. Sivasubramanian, and J. Bird. 1993. Regional distribution, insecticide resistance, and reciprocal crosses between the 'A' and 'B' biotypes of *Bemisia tabaci*. *Insect Sci. Appl.* 14: 127–138.
- DeBarro, P. J. 1995. *Bemisia tabaci* biotype B: a review of its biology distribution and control. Technical Paper, Division of Entomology, Commonwealth Scientific and Industrial Research Organisation, Canberra, Australia.
- DeBarro, P. J., and P. J. Hart. 2000. Mating interactions between two biotypes of the whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Australia. *Bull. Entomol. Res.* 90: 103–112.
- DeBarro, P. J., F. Driver, J. W. H. Trueman, and J. Curran. 2000. Phylogenetic relationship of world populations of *Bemisia tabaci* (Gennadius) using ribosomal ITS1. *Mol. Phylogenet. Evol.* 16: 29–36.
- Dobzhansky, T. 1937. *Genetics and the origin of species*. Columbia University Press, New York.
- Frohlich, D. R., I. Torres-Jerez, I. D. Bedford, P. G. Markham, and J. K. Brown. 1999. A phylogeographical analysis of the *Bemisia tabaci* species complex based on mitochondrial DNA markers. *Mol. Entomol.* 8: 1683–1691.
- Gennadius, P. 1889. Disease of tobacco plantations in the Trikonika. The aleyrodid of tobacco. *Ellenike Georgia* 5: 1–3.
- Gunning, R. V., F. J. Byrne, and A. L. Devonshire. 1997. Electrophoretic analysis of non-B and B-biotype *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in Australia. *Aust. J. Entomol.* 36: 245–249.
- Las, A. 1979. Male courtship persistence in the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae). *Behaviour* 72: 107–125.
- Li, T.-Y., and U. Maschwitz. 1985. Sexual behavior in the whitefly *Trialeurodes vaporariorum* Westw. *Acta Entomol. Sin.* 28: 233–236.
- Li, T.-Y., S. B. Vinson, and D. Gerling. 1989. Courtship and mating behavior of *Bemisia tabaci* (Homoptera: Aleyrodidae). *Environ. Entomol.* 18: 800–806.
- Maruthi, M. N., J. Colvin, R. M. Thwaites, G. K. Banks, G. Gibson, and S. E. Seal. 2004. Reproductive incompatibility and cytochrome oxidase I gene sequence variability amongst host-adapted and geographically separate *Bemisia tabaci* populations (Hemiptera: Aleyrodidae). *Syst. Entomol.* 29: 560–568.
- Matthews, R. W., and J. R. Matthews. 1978. *Insect behavior*. Wiley, New York.
- Mound, L. A., and S. H. Halsey. 1978. *Whitefly of the world*. British Museum of Natural History, Chichester, and Wiley, London, United Kingdom.
- Oliveira, M. R. V., T. J. Henneberry, and P. Anderson. 2001. History, current status, and collaborative research projects for *Bemisia tabaci*. *Crop Prot.* 20: 709–723.
- Nirgianaki, A., G. K. Banks, D. R. Frohlich, Z. Veneti, H. R. Braig, T. A. Miller, I. D. Bedford, P. G. Markham, C. Savakis, and K. Bourtzis. 2003. *Wolbachia* infections of the whitefly *Bemisia tabaci*. *Curr. Microbiol.* 47: 93–101.
- Perring, T. M. 2001. The *Bemisia tabaci* species complex. *Crop Prot.* 20: 725–737.
- Perring, T. M., A. D. Cooper, and D. J. Kazmer. 1992. Identification of the poinsettia strain of *Bemisia tabaci* (Homoptera: Aleyrodidae) on broccoli by electrophoresis. *J. Econ. Entomol.* 85: 1278–1284.
- Perring, T. M., A. D. Cooper, R. J. Rodriguez, C. A. Farrar, and T. S. Bellows, Jr. 1993. Identification of a whitefly species by genomic and behavioral studies. *Science (Wash., DC)* 259: 74–77.
- Petit-Marty, N., M. T. Vera, G. Calcagno, J. L. Cladera, D. F. Segura, A. Allinghi, M. Rodriguez, P. Gómez Cendra, M. M. Viscarret, and J. C. Vilardi. 2004. Sexual behavior and mating compatibility among four populations of

- Anastrepha fraterculus* (Diptera: Tephritidae) from Argentina. Ann. Entomol. Soc. Am. 97: 1320–1327.
- Price, J. F., D. J. Schuster, and D. E. Short. 1987. Managing sweetpotato whitefly. Greenhouse Grower (December): 55–57.
- Ronda, M., A. Adán, D. F. Beitia, D. Cifuentes, and J. L. Cenis. 2000. Interbreeding between biotypes of *Bemisia tabaci*. European Whitefly Studies Network Newsletter, #3 (http://www.whitefly.org/EWSN-NewDownLds-pdf/EWSN_Newsletter03.pdf).
- Simon, R., J. L. Cenis, S. Demichelis, C. Rapisarda, P. Caciagli, and D. Bosco. 2003. Survey of *Bemisia tabaci* (Hemiptera: Aleyrodidae) biotypes in Italy with the description of a new biotype (T) from *Euphorbia characias*. Bull. Entomol. Res. 93: 259–264.

Received 19 August 2005; accepted 27 December 2005.
